

A New Report of *Prionospio kirrae* (Annelida: Spionidae) from Korea

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ABSTRACT

Spionid polychaete *Prionospio kirrae* Wilson, 1990 is newly reported from the Yellow Sea in Korea. This species is characterized by four pairs of branchiae, which are apinnate on chaetigers 2–4 and pinnate on chaetiger 5, a caruncle extending to the posterior end of chaetiger 1, the presence of a distinctly high dorsal crest on chaetiger 11, and the presence of tridentate hooded hooks with rounded apical teeth. Sequences of partial mitochondrial cytochrome *c* oxidase subunit I (*COI*), 16S ribosomal DNA (16S rDNA), and the nuclear 18S ribosomal DNA (18S rDNA) of the species are determined from Korean specimens.

Keywords: Polychaeta, morphology, molecular analysis, taxonomy, Yellow Sea

INTRODUCTION

Prionospio Malmgren, 1867 is one of the specious groups of spionids, which are commonly found in intertidal to deep sea localities (Dagli and Çinar, 2009). These worms are characterized by a prostomium that is typically broadly rounded to truncate anteriorly, and the presence of branchiae that are either apinnate or pinnate (or combinations of these) on the anterior body (Peixoto and Paiva, 2020). Sigvaldadóttir (1998) placed the species of *Apoprionospio* Foster, 1969 back into *Prionospio* *sensu lato* and some authors followed this suggestion (e.g., Radashevsky, 2015). To date, more than 110 *Prionospio* species have been described worldwide (Peixoto and Paiva, 2020), among which, 11 species (*P. bocki* Söderström, 1920; *P. caspersi* Laubier, 1962; *P. depauperata* Imajima, 1990; *P. elongata* Imajima, 1990; *P. japonica* Okuda, 1935; *P. krusadensis* Fauvel, 1929; *P. membranacea* Imajima, 1990; *P. multibranchiata* Berkeley, 1927; *P. paradisea* Imajima, 1990; *P. pulchra* Imajima, 1990; and *P. saccifera* Mackie & Hartley, 1990) have been recorded in Korean fauna (Paik, 1989; Jung et al., 1998; Song et al., 2017; Lee et al., 2018, 2020).

Prionospio kirrae Wilson, 1990 was originally described from the western Bass Strait to Tasmania, Southern Australia, based on anterior fragments only. Subsequently, this

species was reported under the name *Apoprionospio kirrae* from the Yellow and South China Seas (Zhou and Li, 2009). Zhou and Li (2009) noted that the Chinese populations of *P. kirrae* showed differences from those of Australian populations in the presence of low dorsal crests after about chaetiger 20 and neuropodial prechaetal lamellae. Recently, the molecular markers have been used to be a highly effective approach for species identification, thereby providing a basis for solving problems associated with species identification (Álvarez-Campos et al., 2017; Park and Kim, 2017; Nygren et al., 2018). Accordingly, providing DNA information of the known species is needed.

In this study, we newly report *P. kirrae* from Korean waters with description and images, and determine sequences of three genomic regions, partial mitochondrial cytochrome *c* oxidase subunit 1 (*COI*), 16S ribosomal DNA (16S rDNA), and nuclear 18S ribosomal DNA (18S rDNA).

MATERIALS AND METHODS

Samples of adult worms were collected from intertidal zones of the Yellow Sea, Korea (Fig. 1), using 500 µm-mesh sieves. The live materials were relaxed in 10% MgCl₂ solution, and morphological characters were observed under a stereomicro-

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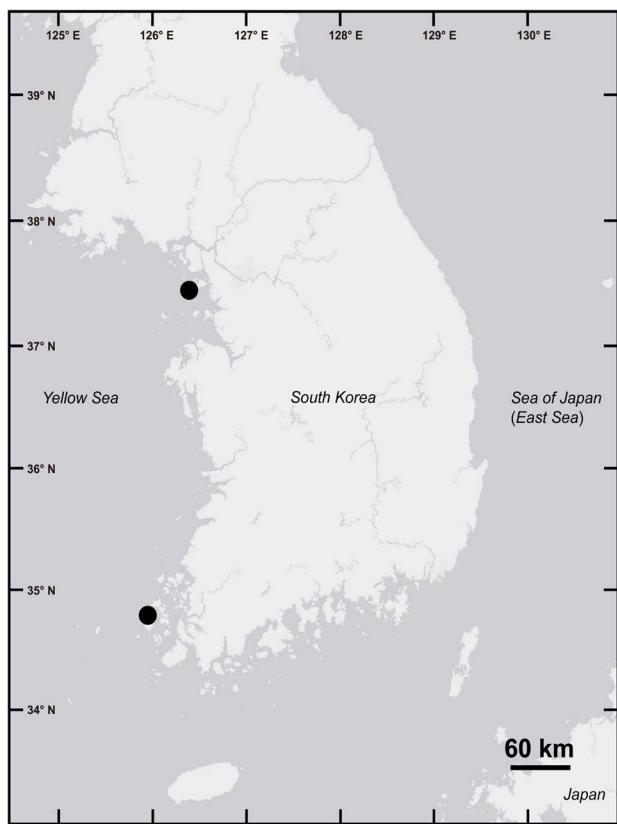


Fig. 1. Map showing the location of the sampling stations (●) where *Prionospio kirrae* Wilson, 1990 were collected in this study.

scope (MZ125; Leica, Germany). Methyl green staining was performed according to the method described by Meißner (2005). Photographs were taken using a Dhyana 400DC digital camera (Tucson, China) incorporating a Mosaic version 15 capture program (Tucson, China). For morphological and genetic studies, materials were fixed in 10% formaldehyde and 95% ethanol, respectively. Specimens used for scanning electron microscopy (SEM) were dehydrated using a t-BuOH freeze dryer (VFD-21S Vacuum Device; Ibaraki, Japan), coated with platinum, and observed using a Hitachi SEM model S-4300SE scanning electron microscope (Hitachi, Japan). All voucher specimens have been deposited at the National Institute of Biological Resources, Korea (NIBR).

Genomic DNA was extracted from a palp of three specimens (NIBRIV0000893858-60) using a LaboPass Tissue Mini kit (Cosmo GENETECH, Seoul, Korea). PCR amplification of three genomic regions was performed according to the method described by Lee et al. (2020), and molecular analyses were performed using sequences aligned with Geneious 8.1.9 (Biomatters, Auckland, New Zealand). A maximum likelihood tree was constructed using MEGA X (Kumar et al., 2018) based on the concatenated sequences of the 16S rRNA and 18S rRNA gene sequences, using GTR + G + I model with 1,000 replicates. GenBank accession numbers of the sequences used for phylogenetic tree construction are listed in Table 1. Newly obtained DNA sequences in this study have been registered at GenBank.

Table 1. GenBank accession numbers and localities for 16S rDNA and 18S rDNA sequences of *Prionospio* species used for the phylogenetic tree

Species	Locality	GenBank accession No.		Reference
		16S rDNA	18S rDNA	
1 <i>P. kirrae</i> Wilson, 1990	Korea	ON310884-6	ON310881-3	Present study
2 <i>P. cf. saccifera</i> Mackie & Hartley, 1990	Japan	LC595702	LC545871	Abe and Sato-Okoshi (2021)
3 <i>P. ehlersi</i> Fauvel, 1928	Southern Ocean	EU340081	EU340095	Mincks et al. (2009)
4 <i>P. cirrifera</i> Wirén, 1883	Southern Ocean	EU340079	EU340093	Mincks et al. (2009)
5 <i>P. variegata</i> Imajima, 1990	Japan	LC595704	LC545873	Abe and Sato-Okoshi (2021)
6 <i>P. lineata</i> Imajima, 1990	Japan	LC595698	LC545868	Abe and Sato-Okoshi (2021)
7 <i>P. membranacea</i> Imajima, 1990	Japan	LC595700	LC545870	Abe and Sato-Okoshi (2021)
8 <i>P. depauperata</i> Imajima, 1990	Korea	MW077202	MW077210	Lee et al. (2020)
9 <i>P. japonica</i> Okuda, 1935	Japan	LC595695	LC545865	Abe and Sato-Okoshi (2021)
10 <i>P. japonica</i> Okuda, 1935	Korea	MW077203-5	MW077211-3	Lee et al. (2020)
11 <i>P. elongata</i> Imajima, 1990	Japan	LC595694	LC545864	Abe and Sato-Okoshi (2021)
12 <i>P. aff. cirrifera</i> Wirén, 1883	Japan	LC595693	LC545863	Abe and Sato-Okoshi (2021)
13 <i>P. sexoculata</i> Augener, 1918	Japan	LC595703	LC545872	Abe and Sato-Okoshi (2021)
14 <i>P. krusadensis</i> Fauvel, 1929	Korea	MW077199	MW077207	Lee et al. (2020)
15 <i>P. krusadensis</i> Fauvel, 1929	Japan	LC595697	LC545867	Abe and Sato-Okoshi (2021)
16 <i>Scolelepis (Scolelepis) daphoinos</i> Zhou, Ji & Li, 2009	Korea	MW494645	MW494652	Lee and Min (2021)



Fig. 2. *Prionospio kirrae* Wilson, 1990, NIBRIV0000893857, with palps, fixed in formalin. Scale bar=1 mm.

SYSTEMATIC ACCOUNTS

Phylum Annelida Lamarck, 1809

Family Spionidae Grube, 1850

Genus *Prionospio* Malmgren, 1867

^{1*}*Prionospio kirrae* Wilson, 1990 (Figs. 2–4)

Prionospio kirrae Wilson, 1990: 251, figs. 16–23.

Apoprionospio kirrae: Zhou and Li, 2009: 117, fig. 1a–h.

Material examined. Yellow Sea, Korea: 4 inds. (anterior fragments), Incheon: Jung-gu, Eurwang-dong, 37°26'50.0"N, 126°22'13.9"E, 15 Jan 2021, intertidal sand, coll. Lee GH; 14 inds. (7 complete and 7 anterior fragments, NIBRIV 0000893856–60), Jeollanam-do: Sinan-gun, Jaeun-myeon, Hanun-ri, 34°55'12.0"N, 126°03'26.2"E, 24 Sep 2021, intertidal muddy sand, coll. Lee GH.

Description. Color opaque white to whitish yellow in formalin-fixed specimens (Fig. 2). Prostomium truncate anteriorly with incision deeply, extending posteriorly to end of chaetiger 1 as caruncle (Fig. 4B); two pairs of reddish eyes arranged in trapezoid, anterior pair crescent-shaped and posterior pair small, rounded (Fig. 3A). Peristomium fused to chaetiger 1 dorsally, not forming lateral wings (Figs. 3A,

4A). Four pairs of branchiae on chaetigers 2–5; apinnate on chaetigers 2–4 and pinnate on chaetiger 5; fourth branchiae up to three times longer than anterior three branchiae, pinnae present on front surface (Figs. 3A, 4A, B). Chaetiger 1 with chaetae on both rami; postchaetal lamellae rounded on both rami, smaller than lamellae on succeeding chaetigers (Fig. 4A). Notopodial postchaetal lamellae foliaceous, largest on chaetigers 2–15, gradually decreasing in size posteriorly. Neuropodial postchaetal lamellae small, rounded in anterior chaetigers, gradually decreasing posteriorly. Prechaetal lamellae present in anterior chaetigers. Prominent dorsal crest on chaetiger 11 (Figs. 3A, 4A) and low crests from chaetiger 20 to almost end of body (Figs. 3B, 4C, D). Interparapodial pouches and ventral flaps absent. Capillary unilimbate, arranged in 3 rows in chaetigers 2–15, becoming one row in succeeding chaetigers. Hooded hooks in neuropodia from chaetiger 16–27 and hooks in notopodia from chaetiger 32–44; hooks with two rounded apical teeth above main fang (Fig. 3D). Ventral sabre chaetae in neuropodia from chaetigers 10–11 (Fig. 3E). Pygidium with thin, long cirrus and one pair of short, thick ventral cirri (Figs. 3C, 4E).

Methyl green staining pattern. Prostomium, peristomium, postchaetal lamellae in anterior chaetigers, and dorsal crest

Korean name: ^{1*}기라예쁜얼굴갯지렁이 (신칭)

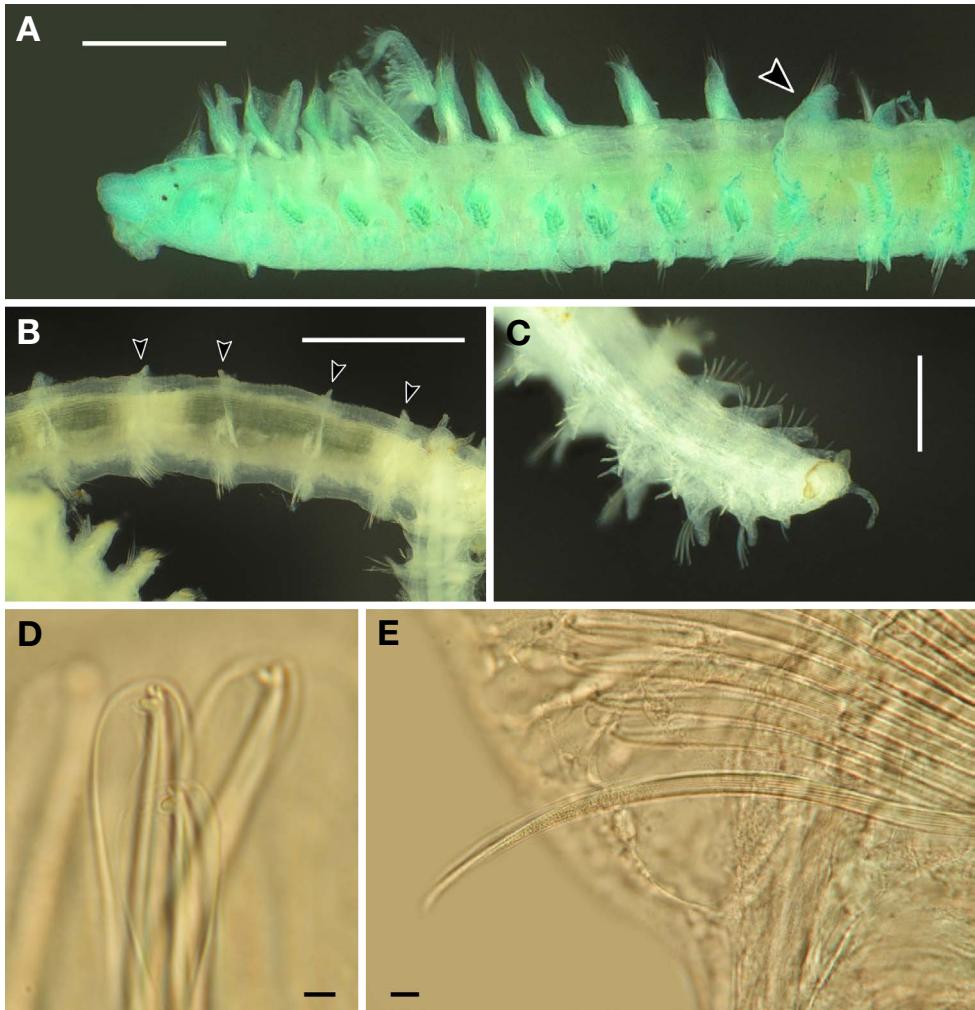


Fig. 3. *Prionospio kirrae* Wilson, 1990, NIBRIV0000893856, palps removed. A, Anterior end with 13 chaetigers stained with methyl green solution, dorsolateral view, arrowhead indicating prominent dorsal crest on chaetiger 11; B, Chaetigers 27–31, dorsal view, arrowheads indicating low dorsal crests; C, Pygidium, ventral view; D, Neuropodial hooded hooks, front-lateral view; E, Ventral sabre chaeta of chaetiger 12. Scale bars: A, B=0.5 mm, C=0.2 mm, D=5 µm, E=10 µm.

on chaetiger 11 most intensely stained (Fig. 3A).

DNA sequence analysis. The sequences of *COI*, 16S rDNA, and 18S rDNA from three specimens of *P. kirrae* were determined [GenBank accession Nos. ON303839–01 for *COI* (up to 677 bp), ON310884–6 for 16S rDNA (up to 519 bp), and ON310881–3 for 18S rDNA (up to 1,791 bp)]. Intra-specific differences for the *COI* (679 bp) and 16S rDNA (519 bp) sequences were 0.5–0.8% and 0.0–0.2%, respectively, whereas no variation was detected in 18S rDNA (1,791 bp). Pairwise genetic distances were calculated between *P. kirrae* and other *Prionospio* species available from GenBank database, and a phylogenetic tree was constructed (Fig. 5). The interspecific genetic distances of *COI* sequences were 21.6–26.7% among *P. kirrae* and other five species: *P. cirrifera*,

P. dubia, *P. ehlersi*, *P. cf. plumosa*, and *P. steenstrupi* (Asha et al., unpublished; Wiklund and Dahlgren, unpublished; Schulze et al., 2000; Aylagas et al., 2016). For 16S rDNA and 18S rDNA, the interspecific genetic distances were 16.8–22.3% and 1.2–4.1%, respectively. In the phylogenetic tree, Korean *P. kirrae* was clearly distinguished from other *Prionospio* species (Fig. 5). The gene sequences determined in this study, along with morphological observations, will provide a valuable basis for further taxonomic or phylogenetic studies on the genus *Prionospio*.

Remarks. The Korean specimens of *P. kirrae* well agree with the original and subsequent descriptions by the following characteristics combined: (1) truncate prostomium with deep incision anteriorly; (2) four pairs of branchiae,

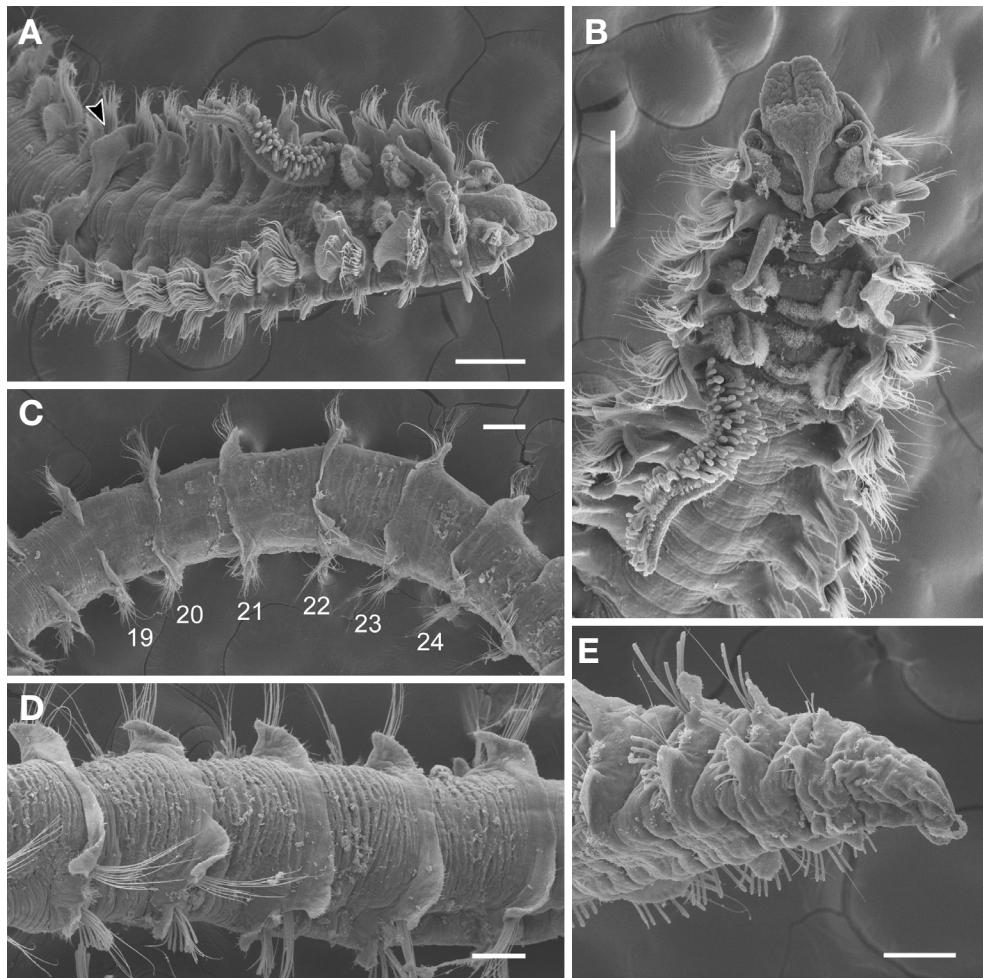


Fig. 4. Scanning electron microscopy images of *Prionospio kirrae* Wilson, 1990, palps and right branchia on the chaetiger 5 removed. A, Anterior end, dorsolateral view, arrow indicating prominent dorsal crest on chaetiger 11; B, Anterior end, dorsal view; C, Chaetigers 18-25, dorsal view; D, Chaetigers 36-40, dorsal view; E, Pygidium, ventrolateral view. Scale bars: A-C=0.2 mm, D, E=0.1 mm.

apinnate on chaetigers 2-4 and pinnate on chaetiger 5, with pinnae present on the front surface; (3) caruncle extending to the posterior end of chaetiger 1; (4) presence of a conspicuously high dorsal crest on chaetiger 11; (5) presence of tridentate hooded hooks with rounded apical teeth (Wilson, 1990; Zhou and Li, 2009). Wilson (1990) described this species with a caruncle extending to the end of chaetiger 2; however, his illustration clearly indicated that the caruncle extends to the end of chaetiger 1. Morphologically, East Asian specimens (China and Korea) differ from the type specimens (Australia) in the presence of low dorsal crests in posterior chaetigers. The type specimens are all anterior fragments, the further examination of materials from the type locality should be conducted based on the complete individuals.

Prionospio kirrae belongs to *P. caspersi* group which is

characterized by having four pairs of branchiae with apinnate first three pairs and pinnae fourth branchiae (Wilson, 1990). Within this group, *P. caspersi* and *P. tridentata* Blake & Kudenov, 1978 have been reported from Japan (Imajima, 1990a, 1990b) and Korea (Song et al., 2017). The Korean specimens of *P. kirrae* differ from *P. caspersi* from Japan with respect to an anteriorly truncate prostomium (vs. bilobed in *P. caspersi*), the presence of a high dorsal crest on chaetiger 11 (vs. chaetiger 7), and tridentate hooded hooks (vs. bidentate) (Imajima, 1990a). The Korean specimens of *P. kirrae* also differ from *P. tridentata* from Japan by the anteriorly truncate prostomium (vs. bilobed in *P. tridentata*), pinnae present on the front surface of the fourth branchiae (vs. posterior surface), the presence of a high dorsal crest on chaetiger 11 (vs. chaetiger 7), and the rounded apical teeth of hooded hooks (vs. pointed) (Imajima, 1990b).

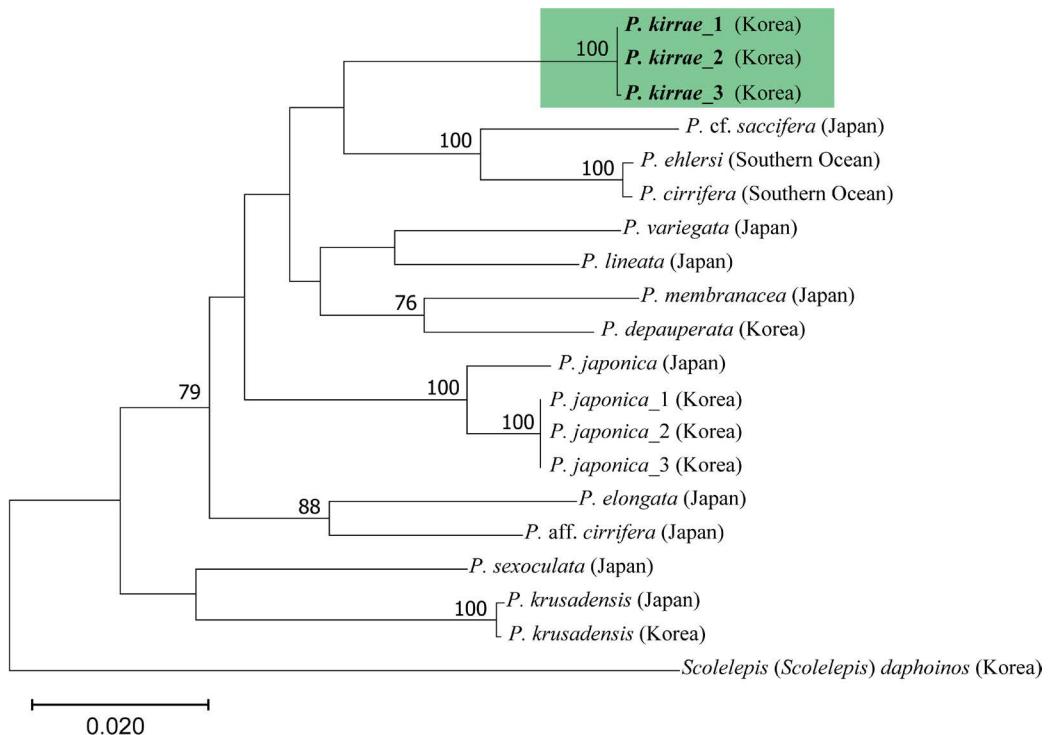


Fig. 5. A maximum likelihood tree inferred from the concatenated partial gene sequences of the 16S rDNA (504 bp) and the 18S rDNA (1,681 bp). The sequences of *Prionospio kirrae* Wilson, 1990 obtained in this study are highlighted in bold. Bootstrap values of >50 as a percentage of 1,000 bootstrap replicates are given at the respective nodes. The gene sequences of *Scolelepis (Scolelepis) daphoinos* Zhou, Ji & Li, 2009 was used as an outgroup taxon.

Habitat. Sand or muddy sand intertidally.

Distribution. Australia (type locality: the Bass Strait to Tasmania, Southern Australia), China (Yellow Sea and South China Sea), Korea (Yellow Sea).

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CONFLICTS OF INTEREST

Gi-Sik Min, contributing editor of the *Animal Systematics, Evolution and Diversity*, was not involved in the editorial evaluation or decision to publish this article. Geon Hyeok Lee has declared no conflicts of interest.

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